Development of microarrays for rapid detection of toxigenic cyanobacteria taxa in water supply reservoirs

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Introduction

Reservoirs are the principal supplies of drinking water for many urban areas and also serve as major recreational areas. In North Carolina, reservoirs are generally characterized by high nutrient levels, high turbidity, high total organic carbon, and low alkalinity. They also commonly develop cyanobacterial blooms which can comprise more than 90% of the phytoplankton cell number at times. Dominant cyanobacteria taxa include *Cylindrospermopsis* spp. (including *Cylindrospermopsis raciborskii*), *Anabaenopsis, Planktolyngbya limnetica, Aphanocapsa* sp., *Aphanizomenon gracile, Oscillatoria, Anabaena, Microcystis,* and *Aphanizomenon*, and microcystins have been found in raw water from these reservoirs.

Materials and Methods

We are developing microarray detection methods for assessment of cyanobacterial taxa in these freshwater systems. In previous work, our prototype arrays were able to detect the DNA of heterotrophic bacteria, eukaryotic protists and cyanobacteria in samples of genomic DNA extracted from three different lakewater samples with a high degree of sensitivity and specificity. To expand the prototype array for cyanobacteria our first step has been to identify cyanobacterial taxa of interest through the literature and field data. Second, cyanobacterial gene libraries were constructed from four representative NC reservoirs using PCR with primers that target cyanobacterial small subunit ribosomal DNA (SSU rDNA) genes. Third, taxon-specific (generally species or clone-specific) PCR primers and 50-mer oligonucleotide probes are designed to complement unique sequences in the variable regions of the SSU rDNA. Fourth, the oligonucleotide probes are printed onto glass slides to form the microarray. Fifth, using both the microarray and real-time PCR, probes are tested for specificity and sensitivity. Finally, the microarray will be used to assess the presence and relative abundance of targeted cyanobacterial taxa over a three year period in NC reservoirs. Microarray results will be compared to direct microscopic count assessments. An additional aspect is that we are also developing oligonucleotide probes for the array that target known toxin biosynthesis genes (e.g. microsytin and cylindrospermopsin synthesis genes).

Conclusion

At present, we are testing our first group of primers and oligonucleotide probes targeting 25 known cyanobacterial taxa (at the species or sub-species level) and 17 novel cyanobacterial clones derived from the NC reservoirs prior to spotting on arrays and field testing. We anticipate that microarrays will be a powerful tool with the potential to assess the abundance of cyanobacterial in near real-time. Such data will aid in management decisions to prevent or mitigate the effects of cyanobacterial blooms.